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PRINCIPAL INVESTIGATOR: Wei-Zen Wei, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, Michigan 48201

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Wei-Zen Wei

E-Mail: weiw@karmanos.org

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14. ABSTRACT

Our goal is to eliminate the tumor by vaccination and local ablation to render long-term immune protection without excessive autoimmune sequelae. Complimenting this regimen is systemic modulation of natural/induced Treg (iTreg) and intratumoral expression of immune augumenting cytokines. The two aims are to
(1) Test the hypothesis that cryosurgery of cytokine enriched tumors amplifies Her-2 vaccine response, and
(2) Test the hypothesis that disabling iTreg conversion enhances Her-2 immunity, not autoimmunity.

In aim 1, we will measure immune response to tumor associated Her-2 by cryotherapy with and without DNA vaccination and the effect of intratumoral expression of cytokine. In aim 2, we will evaluate the degree of iTreg conversion in tumor bearing mice, measure Her-2 vaccine response in Her-2+TIEG1-/- mice which do not generate iTreg, and measure the induction of experimental autoimmune thyroiditis in iTreg deficient mice.

15. SUBJECT TERMS
None provided.

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INTRODUCTION

Our goal is to eliminate the tumor by combining vaccination with local ablation to render long-term immune protection without excessive autoimmune sequelae. Complimenting this regimen is systemic modulation of regulatory T cells (Treg) and intratumoral expression of immune augumenting cytokines. We are testing two related hypotheses in the specific aims.

Aim 1 Test the hypothesis that cryosurgery of cytokine enriched tumors amplifies Her-2 vaccine response.

Aim 2 Test the hypothesis that disabling iTreg conversion enhances Her-2 immunity, not autoimmunity

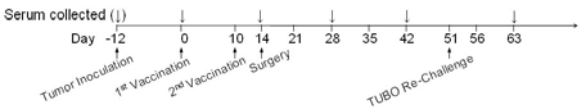
BODY

Aim 1 Test the hypothesis that cryosurgery of cytokine enriched tumors amplifies Her-2 vaccine response.

<u>Cryoablation or surgical resection of neu+ mammary tumor elevates vaccine response to reject a second tumor challenge</u>

Tumor cryoablation, the use of freezing temperatures to destroy tumor tissue while preserving tumor-associated antigens, is used to ablate breast cancer lesions in selected cases. There is controversy on whether cryoablation by itself induces or amplifies tumor immunity. We tested the hypothesis that cryosurgery of the tumor amplifies vaccine induced HER-2 immunity. Wild type BALB/c mice were inoculated with neu+ TUBO tumor before they received vaccination and cryoablation (Fig. 1). All tumors regressed whether mice received vaccination, cryoablation or both (Fig. 2). Significantly higher levels of anti-neu antibodies were produced in mice receiving both vaccination and cryoablation, supporting enhanced immune response by cryoablation in the presence of a strong vaccine response (Fig 4). Full protection against tumor re-challenge was observed in vaccinated mice and partial protection in un-immunized mice whose tumors were removed by cryoablation (Fig.3), indicating immune activation by tumor growth and cryotherapy. Studies in BALB NeuT mice with immune tolerance to neu is underway to determine the conditions necessary to achieve similar immune enhancement by local ablation.

Figure 1. Experimental scheme –BALB/c mice



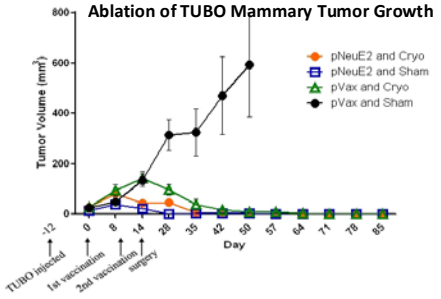


Figure 2. DNA vaccination-cryoablation combination therapy. BALB/c mice inoculated with neu(+) TUBO mammary tumor in #4 mammary fat pad received electro-vaccination with pNeuE2 or control plasmid pVax when tumors reached ~4x4mm. Vaccination was repeated 10 days later. On day 14, mice either underwent cryoablation or sham surgery. Tumor growth was measured weekly.
Conclusion: TUBO tumors regress whether mice received pNeuE2 vaccinatino or cryoablation or the combination of both.

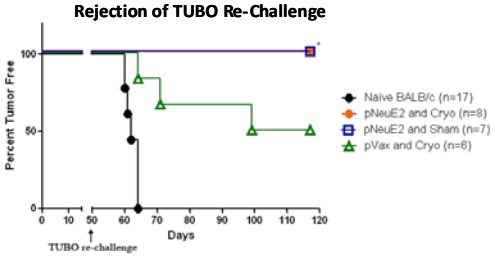


Figure 3. Rejection of TUBO re-challenge. All mice in Figure 2 with complete tumor regression were re-challenged with TUBO cells in mammary gland #9 (right flank) on day 51. *p<0.05 when compared with pVax/cryo group (Wilcoxon test).

Conclusion: pNeuE2 vaccinated mice were protected from TUBO re-challenge whether primary tumors regressed by vaccination alone or by vaccination plus cryotherapy. Cryoablation alone rendered partial protection against a re-challenge.

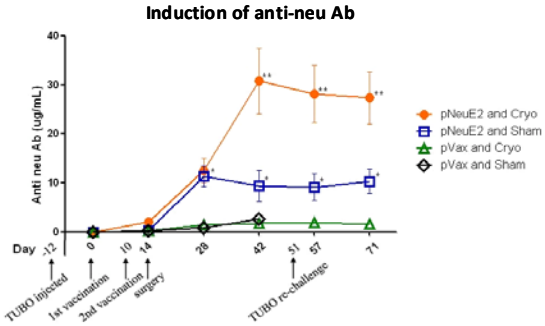


Figure 4. Cryoablation enhanced Ab response to vaccination. Blood samples were collected at two week intervals for anti-neu Ab analysis. **p<0.02, pNeuE2/Cryo vs. pNeuE2/Sham *p<0.05, pNeuE2/Sham vs. pVax/Cryo. (unpaired t-test).

Conclusion: Mice receiving a combination of pNeuE2 and cryoablation had a significant increase in anti-neu Ab levels compared to either treatment alone.

Aim 2 Test the hypothesis that disabling iTreg conversion enhances Her-2 immunity, not autoimmunity

Assessing the balance between tumor immunity and opportunistic autoimmune disorders—A notable side effect of fostering anti-tumor immunity by systemic immune modulation is the promotion of autoimmune symptoms where the highly prevalent autoimmune thyroid disease is prominent. Autoimmune thyroiditis is strongly influenced by *HLA/H2* haplotype and suppressed by regulatory T cells (Tregs) (reportable outcome #3). We have established several test systems to evaluate concurrent induction of tumor immunity and experimental autoimmune thyroiditis (EAT) in MHC-variable strains differing in EAT susceptibility. In this report, we examined (EAT)-susceptible CBA/J *($H2^k$)* mice in which strong tumor immunity could be induced by Treg depletion and immunization with irradiated tumor cells from a spontaneous CBA mammary adenocarcinoma line (A22E-j). When induction of EAT with mouse thyroglobulin (mTg) was combined with induction of tumor immunity under the umbrella of Treg depletion, EAT was enhanced in mice protected from lethal tumor challenge.

KEY RESEARCH ACCOMPLISHMENTS

1. Established immune enhancement by cryoablation after inducing a strong anti-tumor immunity by DNA vaccination in wild type mice.
2. Established a test system to evaluate the balance between tumor immunity and autoimmunity in mice that are susceptible to develop experimental autoimmune thyroiditis and to show the increased risk of EAT by systemic immune modulation.

REPORTABLE OUTCOMES

Venuprasad, P., and Wei, WZ. Modulation of HER-2 DNA vaccine response by tumor cryosurgery and abrogation of inducible regulatory T cells (iTreg) conversion. DOD Breast Cancer Program, Era of Hope meeting, 2011.

Veenstra, J, Littrup, P, Wei, WZ. Amplification of tumor immunity with cryoablation. Proc. AACR, 2011.

Kong, Y.M., Brown, N.K., Flynn, J.C., McCormick, D.J., Brusic, V., Morris, G.P. and David, C.S. Efficacy of *HLA-DRB1*03:01* and *H2E* transgenic mouse strains to correlate pathogenic thyroglobulin epitopes for autoimmune thyroiditis. *J. Autoimmunity*. **37**:43-70, 2011.

CONCLUSIONS

Tumor destruction by local ablation enhances tumor immunity when anti-tumor response is first installed by prior immunization. The observation in wild type mice is being further tested in mice with immune tolerance to tumor associated antigen. In CBA/J mice susceptible to the development of autoimmune thyroiditis, induction of tumor immunity by systemic Treg depletion elevated the severity of autoimmunity. This autoimmune susceptible model system will be effective for evaluating autoimmune risk of novel immunotherapy regimen.